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We have developed the hardware and software to evaluate stress and strain in patch-clamped membranes. We found that there is lipid flow during stress in the patch and therefore it is not under significant steady state stress and stress must be transferred through the cytoskeleton. We have also developed techniques to study the ultrastructure of the patch using high voltage electron microscopy.

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The original aims of the grant were the study of the effect of cytoskeletal reagents on the activty of stretch activated ion channels.

We found the main difficulty in characterizing the responsiveness was a wide scatter in the dose-response curves. The curves are sigmoidal and we found wide variability between the pressure needed to reach the midpoint oif the curve and also in the maximal slope of the curve (sensitivity). In order to reduce the scatter, we decided that we must begin a program of visualizing the patch so that we could actually measure the stress and strain rather than having to rely on the applied pressure as the independent variable.

We built a high resolution optical setup in which to do patch clamping. To work at high magnification, we had to redesign the manipulators for minimal drift, redesign the manipulator controller for minimal vibration, and redesign the condenser and illuminator for water immersion, arc-lamp, illumination. We had to build special low clearance tissue culture chambers to permit nearly horizontal approach. We had to design and build hardware and software to acquire data off SVHS video tapes with known time synchronization to pressure stimulii. We wrote software to extract digitized images of the patch and software to curve fit the images to geometric models of the patch so that we knew the patch area, radius of curvature and attachment point to the glass pipette. We also had to design and build a high speed pressure clamp to apply controlled peressure steps or ramps to the patch. We also worked out methods to measure changes in the patch capacitance during stretch. With these tools in place, we measured the channel activity, patch capacitance, membrane stress and membrane strain as a function of time following a step in pressure. The results indicated that the much of the scatter disappears when data is plotted as a function of membrane tensions rather than as a function of applied pressure. We also found that the membrane is rather soft with elasticities of ca. 20dyn/cm indicating that we were not stressing the lipid membrane since that would have produced much higher values. Additionally we found the stress induced increase of specific capacity to be about $0.6uF/cm^2$, and independent of membrane area. This capactiance is characteristic of pure lipids, not protein containing membranes which are more in the range of $1.2\ uF/cm^2$. Preliminary experiments have indicated that treatment with cytochalasins do not affect any of the observed parameters, contrary to expectations.

During tenure of the grant we also began a study of the ultrastructure of the patch in order ot better understatnd the distribution of stress and the effectrs of cytoskeletal reagents. We developed an entirely new approach using the high voltage electron microscope. This work also required the development of new apparatus and techniques for handling patch pipettes. We now quick freeze the patch, freeze dry it, and examine the whole mount (in the pipette tip) in the HVEM. We are able to visualize cytoskeleton, membrane and what appear to be intramembrane particles. Using colloidal gold labels we can visualize the location of cell membrane receptors such as acetylcholine receptors and lectin binding sites and elements of the cytoskeleton such as actin and spectrin.

In order to try and measure the change in dimensions of an SA channel associated with the gating step, we built a laser interferometer which can meassure changes in position of much less than an Angstrom. This device has not yet been used on the patch,but ht ebasic idea is to cross correlate the channel openings with the scattered light signal.

We also examined a number of different tissues for stretch activated ion channels and found a whole class of SA channels in astrocytes from the rat. We also found a gadolinium to be a useful blocker of non-selective cation SA channels.

The grant supported some theoretical work on SA channels and acoustical transduction which has appeared in several review papers.

Contract # DAAG29-85-K-0135

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